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Diet and trophic niche space and overlap of Lake Ontario salmonid species using stable isotopes and stomach contents

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ABSTRACT

Lake Ontario supports a diversity of native and non-native salmonids which are managed largely through stocking practices. Ecological changes (e.g., invasive species) altering the food web structure accompanied with shifts in prey abundance, necessitate understanding the trophic niches of Lake Ontario salmonids to aid in management. The objectives of this study were to quantify salmonid (5 species) trophic niches and dietary proportions using stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of a large sample set (adult fish (>300 mm; $n = 672$) and key offshore prey (5 species, $n = 2037$) collected across Lake Ontario in 2013. Estimates of prey based on stable isotope ratios were similar to stomach contents. Based on stable isotope ratios, non-native prey dominated salmonid diet; in particular alewife (*Alosa pseudoharengus*) constituted the majority (0.31 to 0.93) of all salmonid diets, and round goby (*Neogobius melanostomus*) contributed 0.26 and 0.19 of brown trout (*Salmo trutta*) and lake trout (*Salvelinus namaycush*) diets, respectively. Trophic niche overlap was high between all salmonids, except lake trout. The largest trophic niche overlap occurred between Chinook (*Oncorhynchus tshawytscha*), coho (*Oncorhynchus kisutch*), and Atlantic salmon (*Salmo salar*), and their reliance on alewife infers a strong pelagic foraging strategy. Lake, brown and rainbow (*Oncorhynchus mykiss*) trout had larger and/or more distinct trophic niches indicative of a more variable diet across individuals and utilizing different foraging strategies and/or habitats. Overall, Lake Ontario salmonids maintained a high reliance on alewife, and their potential for plasticity in diet provides important information to management regarding population sustainability.

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Introduction

Great Lakes agencies began stocking non-native Pacific salmon in the late 1960s in response to historic low catch rates of native salmonids and nuisance levels of non-native alewife (*Alosa pseudoharengus*) that were rapidly expanding in the absence of predator controls (Dettmers et al., 2012). As a result, Lake Ontario, the smallest Great Lake by area yet most densely populated within its basin, now supports a large and diverse salmonid community with non-native Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), as well as re-introduced native lake trout (*Salvelinus namaycush*) and

Atlantic salmon (*Salmo salar*). Collectively these fishes serve as the apex predators in the offshore areas (depths >30 m) of Lake Ontario, consuming large biomasses of prey fishes and exerting a cascading food web effect on lower trophic levels (Jones et al., 1993; Mills et al., 2003; Stewart et al., 2013). These salmonids, particularly Chinook salmon, are part of a large and economically important Lake Ontario recreational fishery that generates an annual economic impact in excess of \$150 million between Canada and the United States of America (U.S.) (Connelly and Brown, 2009; DFO, 2012). To sustain the salmonid community and fishery, Canadian and U.S. resource managers coordinate stocking of more than five million trout and salmon in Lake Ontario annually (NYSDEC, 2016; OMNRF, 2016). However, on-going ecological change and unanticipated high levels of naturalization among the stocked salmonid populations (Claramunt et al., 2012; Connerton et al., 2009) have prompted resource managers to re-consider current stocking practices in light of emerging predator-prey imbalances (Brenden et al., 2012). Management decisions around stocking must account for anticipated survival of stocked fish (Coghlan et al., 2007;

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Lantry et al., 2011), natural reproduction (Connerton et al., 2009; Nack et al., 2011), and prey supply (Jones et al., 1993; Murry et al., 2010) in order to maintain a suitable predator-prey balance to support ecosystem health and productive fisheries (Dettmers et al., 2012; Stewart et al., 2013).

With ecological change (e.g., invasive species, climate change, etc.) altering the structure and efficiency of the Laurentian Great Lakes food webs (Bunnell et al., 2014; Mills et al., 2003), there is a need for a more holistic approach to resource management and to better understand the impacts on stocked salmonids. Shifts in composition and abundance of predator and prey have important implications for fishery management decision makers (Jones et al., 1993; Stewart et al., 2013). For instance, although stocking non-native Pacific salmon achieved the initial goal of reducing non-native alewife populations (Crawford, 2001; Dettmers et al., 2012), there is recent concern that prey fish stocks may not be adequate to support the economically important salmonid fishery (Brenden et al., 2012; He et al., 2015). Recent invasive species such as dreissenid mussels and round goby (*Neogobius melanostomus*) have generated food web changes that are not fully understood, specifically the impacts to top predators (Rush et al., 2012; Vanderploeg et al., 2010 – cited in Dettmers et al., 2012). Recent research has begun to elucidate the trophic structure and niches of the offshore prey fish community (Mumby et al., 2018; Rush et al., 2012) and, to an extent, top predators (Yuille et al., 2015) since the appearance of these recent invasive species but much remains to understand how this may affect salmonids. Given the ecological and economic importance of salmonids in the offshore of Lake Ontario, surprisingly few studies have directly compared the diet and feeding relationships of these coexisting species.

The offshore prey fish community of Lake Ontario is relatively simple and the potential for dietary overlap among salmonids in Lake Ontario is high. The offshore prey fish community consists mainly of alewife, rainbow smelt (*Osmerus mordax*), round goby, slimy sculpin (*Cottus cognatus*) and deepwater sculpin (*Myoxocephalus thompsonii*). Based on past studies using stomach contents, all salmonid species in Lake Ontario primarily consume the abundant alewife with lesser amounts of the other species (Brandt, 1986; Rand and Stewart, 1998). Relative to other salmonids in Lake Ontario, lake trout consume a variety of offshore prey fishes including alewife, rainbow smelt and sculpins (Brandt, 1986; Kiriluk et al., 1995; Rand and Stewart, 1998) and have recently been reported to consume round goby (Colborne et al., 2016; Dietrich et al., 2006; Rush et al., 2012). Brown trout also consume a more diverse diet with a mix of nearshore and offshore prey (Brandt, 1986; Rand and Stewart, 1998). Although rainbow trout consume high proportions of alewife, they also have a varied diet, consuming not only fish, such as rainbow smelt but also a notable abundance of invertebrates, and feed across multiple trophic levels (Brandt, 1986; Negus and Hoffman, 2013; Roseman et al., 2014). Chinook salmon are thought to be obligate pelagic predators with a less diverse diet (Jacobs et al., 2013; Roseman et al., 2014), and may be more vulnerable to changes in pelagic prey stocks (e.g., alewife), than top predators with a more diverse diet, such as lake trout and brown trout. Similarly, coho salmon are known to primarily feed on the pelagic alewife and rainbow smelt (Brandt, 1986), yet can have a more varied diet dependent on food availability (Roseman et al., 2014). Little is known about Atlantic salmon given their extirpation from Lake Ontario over 100 years ago; however, Atlantic salmon target offshore prey in other freshwater systems (Ketola et al., 2000; Kirn and Labar, 1996; Roseman et al., 2014). Among the salmonids, there is a strong potential for dietary overlap from consuming alewife, however, the ecological changes in prey abundances and invasive species occurring in Lake Ontario may potentially be altering these dietary relationships.

Past salmonid diet studies have relied on stomach content analyses to describe feeding preferences (e.g., Brandt, 1986; Rand and Stewart, 1998; Roseman et al., 2014), but they only provide a snapshot of the most recent feeding event. As such, more recent studies have started combined stomach contents and stable isotope analyses of salmonids

in the Great Lakes (e.g., Harvey and Kitchell, 2000; Negus and Hoffman, 2013; Yuille et al., 2015). Stable isotope ratios (typically $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) provide a complementary approach that reflects the time-integrated signature of energy sources assimilated into tissues, reflecting periods from days to years depending on the tissue and size of the organism (Peterson and Fry, 1987; Pinnegar and Polunin, 1999; Post, 2002; Thomas and Crowther, 2015; Vander Zanden et al., 2015). This longer-term integration of assimilation of prey sources provides a more accurate reflection of the trophic interactions among predators and their prey than stomach content analysis alone given the environmental heterogeneity experienced by most predators. Isotope mixing models allow for the quantification of dietary proportions and Bayesian mixing models are being used more frequently when describing diet compositions (Parnell et al., 2010; Phillips, 2012; Phillips et al., 2014). Stable isotope analysis has also been used to describe and make broad ecological inferences on the trophic niche of a species (Bearhop et al., 2004; Layman et al., 2007), and hereafter our references to trophic niches are determined by stable isotopes. The trophic niche space occupied by a species can be estimated from the total standard ellipse area (SEA) encompassed by individuals in $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ bi-plot (Jackson et al., 2011; Layman et al., 2007). Combining the strengths of time-integrated assimilated isotope signatures with stomach contents to describe and compare diet and trophic niche properties has provided ecologists with the tools needed to discern and describe key factors driving community structure.

With improved analytical methods to assess current comparative and quantitative information on feeding ecology of salmonid species in the Great Lakes, the objective of this study was to quantify diet and size and overlap of the trophic niche for salmonid species in Lake Ontario. On-going changes in the composition and abundance of the prey fish community (Lantry et al., 2007; Walsh et al., 2014; Weidel and Walsh, 2015), mounting evidence that naturalized populations may be reducing the ability to manage predators solely through stocking (Connerton et al., 2009; Nack et al., 2011), sustained interest in maintaining the economic benefits of a fishery (Dettmers et al., 2012; Stewart et al., 2013), and on-going need to rehabilitate native species (Stewart et al., 2013; Lantry et al., 2014) combine to necessitate understanding the trophic niches of the Lake Ontario salmonids to aid in management. This research extends beyond Yuille et al. (2015) in that we aim to better understand salmonid dietary proportions as both stomach contents and isotopic mixing models are utilized in concert with isotopic niche space. Also, additional salmonids (i.e., Atlantic salmon) and larger sample sizes exclusive to a single year of sampling make this research more robust. We hypothesize that given the current composition of the prey fish community and knowledge of diet preferences of salmonids in Lake Ontario: i) salmonid diets are composed largely of alewife and a high amount of trophic niche overlap will occur among salmonid species, and ii) species consuming a more varied diet, like lake trout and rainbow trout, will have less overlap with the other salmonids.

Methods

Fish collection

Adult salmonids (Atlantic salmon, Chinook salmon, coho salmon, brown trout, lake trout, and rainbow trout) and corresponding prey fishes (alewife, rainbow smelt, round goby, deepwater sculpin, and slimy sculpin) were collected from Lake Ontario in 2013. Fishes were collected from April to December 2013, with additional Atlantic salmon samples incorporated from 2008 to 2011 creel surveys due to low sample sizes in 2013. All salmonids analyzed for this study were “adults” > 300 mm fork length. Salmonid samples were obtained primarily from creel surveys, with additional samples coming from agency index gillnet programs. Prey fishes were obtained from gillnets and bottom trawls. Gillnets were fished horizontally on the bottom (graded mesh, monofilament 19-mm to 152-mm) or vertically (surface to

30 m depth, monofilament mesh ranging from 19-mm to 39-mm). Additional description of gear and programs can be found in NYSDEC (2016) and OMNRF (2016). Depths of collection of the fishes ranged from 1 to 175 m with approximately half the effort in Canadian waters and half in the U.S. (Fig. 1).

During fish processing, adult salmonids and prey fishes were identified to species, weighed (g) and both total length (mm) and fork length (mm) were measured. A skinless, boneless, dorsal muscle sample was removed from each fish for stable isotope analysis. For creel caught salmonids, the isotope sample was obtained using a Unicore 3.5 mm biopsy punch (Ted Pella, Redding, CA, U.S.). All stable isotope samples were placed in 2 ml cryovials, frozen, and freeze dried for 48 h at -48°C under a vacuum pressure of 133×10^3 mbar in preparation for stable isotope analysis. For all salmonids, the stomach was removed, placed in 85% ethanol and frozen until later analysis.

Stable isotope analysis

After freeze drying, muscle samples were crushed into a fine powder. Salmonid muscle samples were lipid extracted due to anticipated high C:N ratios (>3.4) while lipids were not removed from prey as these prey typically have lower C:N ratios (≤ 3.4) (Post, 2002). Salmonid samples were lipid extracted using a chloroform:methanol extraction following the Bligh and Dyer (1959) method and was not quantitative for lipids. Both bulk and lipid extracted samples from the same salmonid ($n = 24$) had C:N ratios compared for lipid extraction efficiency. Mean (\pm SD) C:N of bulk samples had high C:N ratios (5.25 ± 2.22) while lipid extracted samples had low C:N ratios (3.28 ± 0.11) indicating that lipids were effectively removed from our samples with the methods used. Both lipid extracted and non-lipid extracted samples were weighed into 5×9 mm tin capsules on a microbalance containing 400 to 600 μg of sample. Combustion of the muscle sample into N_2 and CO_2 gases was executed using an elemental analyzer (Costech, Valencia, CA, U.S.), and a Thermo Finnigan Delta V mass spectrometer (Thermo Finnigan, San Jose, CA, U.S.) to measure the relative abundances of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) within each sample. Standard delta notation (δ) was used to express stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios in parts per thousand (‰) differences from a standard reference material as the following equation: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}} - 1)] \times 1000$ where $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively (Fry, 1991; Hobson and Clark, 1992). The C:N was in mass ratios. Standard reference materials were Pee Dee Belemnite for carbon and atmospheric nitrogen for nitrogen. National Institute of Standards and Technology (NIST) standards were used to calculate the accuracy of the analysis. NIST standards used for $\delta^{13}\text{C}$ were sucrose (NIST 8542)

and L-glutamic acid (NIST 8573), and for $\delta^{15}\text{N}$ were ammonium sulphate (NIST 8548 and 8547) and L-glutamic acid (NIST 8573) ($n = 96$ for each). NIST standards deviated from the certified values by $\leq 0.1\%$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Analytical precision was based on the internal lab standard tilapia and bovine liver (NIST 1577c) ($n = 318$) and had a standard deviation of $<0.1\%$ for $\delta^{13}\text{C}$ and $<0.2\%$ for $\delta^{15}\text{N}$.

Salmonid stomach content analysis

Stomach contents were quantified by total volume (% per prey item), by first determining the mass of the stomach contents (by volume displacement, assuming a density of 1 g/ml) and then sorting the contents to the lowest taxonomic level, assigning proportions to those groupings, and where possible measuring the length of the prey item. Prey proportions to relative weight of stomach contents were determined per species by summing the relative weight of each prey item of all fish of a species over the total weight of the species' stomach contents. Invertebrates ($<2\%$ of the prey proportions) were excluded from our mixing models as they were not frequent in the salmonid diet (even more so when split between terrestrial, pelagic and benthic invertebrates), as well as for the purposes of focusing on prey fish. Empty stomachs were not included in the analysis. Note that creel surveyed salmonids can have a higher occurrence of empty stomachs (e.g., Diana, 1990) which would reduce prey species occurrences in the stomach contents.

Statistical analysis

The trophic niche of the salmonids was determined using Stable Isotope Bayesian Ellipses in R (SIBER) in the package SIAR (Stable Isotope Analyses in R) v.4.2 (Parnell and Jackson, 2013) in R v.3.0.2 (R Core Development Team, 2017). SIBER uses a multivariate ellipse-based approach to compare groups of differing sample sizes (Jackson et al., 2011). Corrected standard ellipse areas (units of $\% ^2$) that contain 40% of the data (SEAc) were calculated to represent the core isotopic niche area (as opposed to the entire niche area by including 100% of the data), correct for small sample sizes, and allow for comparison of differing sample sizes between species (Jackson et al., 2011; Jackson et al., 2012). A Bayesian iterative process was used to create a SEAc estimate for each species based on a subsample of the population's stable isotope values and accounting for uncertainty in the sampled data (Jackson et al., 2011). Based on these estimates, fraction overlap (%) among the species' SEAc was used to express similarities and differences in isotopic niche space utilization.

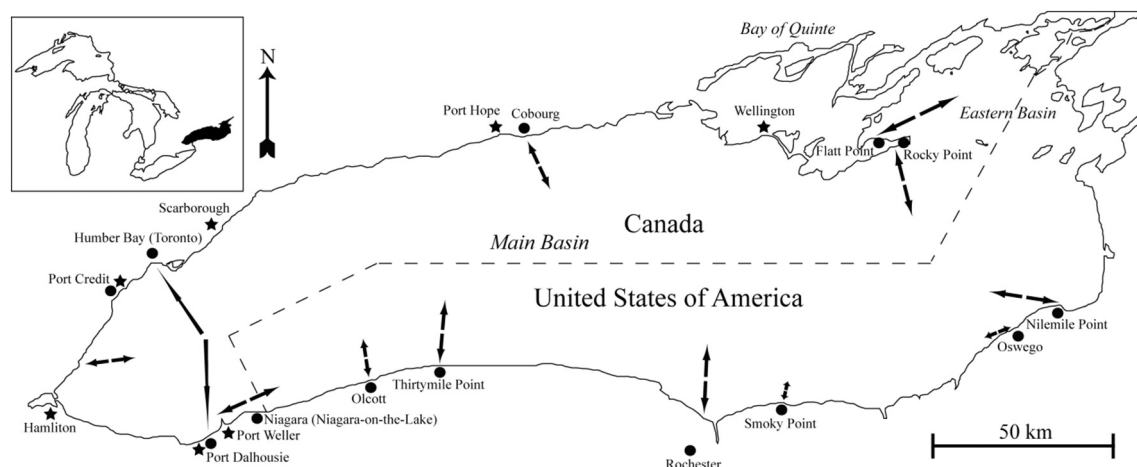


Fig. 1. Lake Ontario salmonid and prey sampling sites completed using 12 different transects (six in the USA and six in Canada) and seven creel survey locations (all in Canada) in 2013. Creel survey locations represented by stars and transects represented by arrows. Transect length and direction represented by arrow length and position.

The relative likely contributions of prey fish to the salmonid diets were determined using a Bayesian mixing model approach also using the SIAR package in R. Any correlations between prey species were identified by diagnostic matrix plots where a decrease in the proportional contribution to the salmonid diet of a prey species caused an increase in proportional contribution of the other prey species due to the requirement for the total dietary contributions to sum to 1. The mean and standard deviation $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the five major offshore prey fish species thought to potentially contribute to salmonid diet were included in the model as sources (i.e., smaller contributions such as invertebrates were not included). The trophic fractionation used in the models were estimated from a diet-tissue discrimination factor (DTDF) based on Chinook salmon described below. For each salmonid species, a model was run for 2×10^5 iterations, with a burn-in of 5×10^4 iterations, and thinning every 15 simulations, and the results are reported using the mean and 95% Bayesian credibility intervals.

As mentioned previously, salmonid isotope samples were lipid extracted if C:N ratios were >3.4 . Although prey species typically have low C:N ratios, the mean C:N ratios for prey species in this study were slightly ≥ 3.4 (Table 1). Lipid extraction can alter the $\delta^{13}\text{C}$ by 1.0–2.0‰, such that if all samples have not been corrected (e.g., prey samples), it can impact the food-web interpretations from isotope mixing models (Murry et al., 2006; Skinner et al., 2016). Thus, all prey samples with a C:N ratio >3.4 , were lipid corrected using the Kiljunen et al. (2006) non-linear mathematical lipid normalization model (as modified by McConnaughey and McRoy (1979) model using different input values) with the Post et al. (2007) percent lipid calculation as recommended by Skinner et al. (2016) and verified with multiple freshwater species. The McConnaughey and McRoy (1979) model used by Kiljunen et al. (2006) is:

$$\delta^{13}\text{C}' = \delta^{13}\text{C} + D \times \left(I + \frac{3.90}{1 + 287/L} \right)$$

where $\delta^{13}\text{C}'$ is the lipid-free δ value; D is 7.018, the difference in carbon isotopic composition between protein and lipid; I is a constant (0.048); and L is the percentage of lipid in the sample of interest. The percent lipid (L) is determined from the Post et al. (2007) percent lipid model:

$$L = -20.54 + 7.24 \times \text{C} : \text{N}$$

where C:N is the C:N ratio of the sample of interest. There was a relatively small difference (range of 0.3 to 1.1‰) between the mean bulk and lipid corrected $\delta^{13}\text{C}$ of prey species (Table 1).

To best estimate the contribution of prey to the salmonid diet, a diet tissue discrimination factor (DTDF) was developed. DTDFs are a key metric for assessing isotopes in food webs and are important in quantifying diet via stable isotope mixing models (Bond and Diamond, 2011).

We estimated DTDF for all salmonids using Chinook salmon. We used Chinook salmon because i) the stomach contents of Chinook salmon >500 mm in Lake Ontario were exclusively alewife, ii) Chinook salmon were the most abundantly sampled predator, and iii) the $\delta^{15}\text{N}$ of Chinook salmon was similar to the other salmonid species (except for lake trout). The lack of diet variation is key in developing a precise estimate of DTDF (Vander Zanden and Rasmussen, 2001). To generate DTDF, we calculated the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for Chinook salmon ≥ 500 mm fork length (mean = 796 mm, $n = 218$) and alewife between 170 and 190 mm total length (mean = 180 mm, $n = 181$). Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of alewife ($\delta^{13}\text{C} = -23.2\text{‰}$, $\delta^{15}\text{N} = 12.7\text{‰}$) was subtracted from mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of Chinook salmon ($\delta^{13}\text{C} = -22.2\text{‰}$, $\delta^{15}\text{N} = 16.1\text{‰}$), respectively, to obtain DTDF values of 1.0‰ for $\Delta^{13}\text{C}$ and 3.5‰ for $\Delta^{15}\text{N}$.

Changes in isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) with body size were assessed for each salmonid species. For each species, a linear regression analyses investigated any changes in isotopic signatures with fork length using R. As we were focused on salmonids >300 mm, larger shifts in diet were not anticipated or observed when plotting the data (as may be expected in juvenile vs. adults) and thus linear relationships were tested. The dietary contributions with body size were then assessed by length classes. For each species, fish samples were binned by 100 mm length increments and individual Bayesian mixing models were run on bins with >10 samples using the model values as indicated above to obtain the estimated prey proportions consumed.

Trophic position (TP) was also calculated for each salmonid species using a one-source model (Vander Zanden et al., 1999):

$$\text{TP}_{\text{consumer}} = \left(\frac{\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}}{\text{DTDF}} \right) + \text{TP}_{\text{baseline}}$$

Generally, the DTDF or trophic increase value is assumed to be 3.4 in $\delta^{15}\text{N}$ between prey and predator (McCutchan et al., 2003; Minagawa and Wada, 1984; Post, 2002; Vander Zanden and Rasmussen, 2001), but the DTDF values calculated above for the Lake Ontario salmonids were used to obtain a better representation of the appropriate salmonid TP ($\Delta^{15}\text{N} = 3.5$). Alewife was used as the baseline organism because alewife comprise a large proportion of salmonid diets (Brandt, 1986; Rand and Stewart, 1998) and were used to calculate salmonid DTDF values. The alewife were assigned TP = 3 because they are secondary/planktivorous consumers (Walsh et al., 2008; Stewart et al., 2009).

Results

Salmonid size and stable isotope data

A total of 672 predators and 2037 prey muscle samples were analyzed for stable isotope ratios from Lake Ontario in 2013. The mean

Table 1
Fork length, stable isotope ratios and C:N ratio (mean \pm SE) of adult (>300 mm) salmonids and prey collected from Lake Ontario between April to December 2013. Atlantic salmon were collected from 2008 to 2011, and 2013. Predator samples were lipid extracted, prey samples were lipid corrected in samples with C:N ratio >3.4 . Both bulk and lipid corrected $\delta^{13}\text{C}$ are indicated for prey. Total length was used for prey.

Species	n	Length (mm)	Bulk $\delta^{13}\text{C}$	Lipid extracted/corrected $\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N
Predator						
Atlantic salmon	41	551 \pm 8		-22.0 \pm 0.1	16.0 \pm 0.1	3.3 \pm 0.0 ^a
Chinook salmon	289	700 \pm 12		-22.1 \pm 0.0	16.2 \pm 0.0	3.4 \pm 0.0
Coho salmon	42	596 \pm 13		-21.9 \pm 0.1	16.1 \pm 0.1	3.3 \pm 0.0
Brown trout	47	517 \pm 15		-21.4 \pm 0.1	15.8 \pm 0.1	3.4 \pm 0.0
Lake trout	127	589 \pm 10		-21.8 \pm 0.1	17.5 \pm 0.1	3.4 \pm 0.0
Rainbow trout	126	593 \pm 8		-22.0 \pm 0.1	15.6 \pm 0.1	3.4 \pm 0.0
Prey						
Alewife	802	140 \pm 1	-24.1 \pm 0.0	-23.1 \pm 0.0	12.6 \pm 0.0	4.0 \pm 0.0
Rainbow smelt	355	110 \pm 1	-23.6 \pm 0.0	-23.0 \pm 0.0	15.0 \pm 0.1	3.5 \pm 0.0
Round goby	448	90 \pm 2	-21.7 \pm 0.1	-21.4 \pm 0.1	13.6 \pm 0.1	3.4 \pm 0.0
Deepwater sculpin	223	126 \pm 2	-24.7 \pm 0.1	-23.6 \pm 0.0	16.9 \pm 0.0	4.1 \pm 0.1
Slimy sculpin	209	97 \pm 1	-24.6 \pm 0.0	-23.7 \pm 0.0	16.6 \pm 0.1	3.8 \pm 0.0

^a Only 2013 samples used, no C:N ratio data available for 2008 to 2011 samples.

(\pm SE) size of salmonid predators ranged from 517 ± 15 mm (brown trout) to 700 ± 12 mm (Chinook salmon) (Table 1). Prey ranged in mean (\pm SE) size (total length) from 90 ± 2 mm (round goby) to 140 ± 1 mm (alewife) (Table 1). The size range of prey of this study is consistent within the size range of prey found in stomachs of salmonids from Lake Huron (Roseman et al., 2014). Both predators and prey were sampled throughout Lake Ontario across bathymetric depths of 1 to 175 m and in all seasons excluding winter. Species differed in capture frequency with bathymetric depth likely owing to species-specific thermal preference. Values of $\delta^{15}\text{N}$ were lowest in rainbow trout ($15.6 \pm 0.1\text{‰}$) (mean \pm SE) and highest in lake trout ($17.5 \pm 0.1\text{‰}$), and spanned a wider range than $\delta^{13}\text{C}$ ($-22.1 \pm 0.0\text{‰}$ in Chinook salmon to $-21.4 \pm 0.1\text{‰}$, in brown trout) (Table 1). For prey species, $\delta^{15}\text{N}$ was lowest in alewife ($12.6 \pm 0.0\text{‰}$) and highest in deepwater sculpin ($16.9 \pm 0.0\text{‰}$), and $\delta^{13}\text{C}$ was relatively similar for most prey species (around -23‰), except for round goby which had a higher $\delta^{13}\text{C}$ ($-21.4 \pm 0.1\text{‰}$) but also a wide range of $\delta^{13}\text{C}$ values (Table 1; Fig. 2; SD = 1.79‰). For more details on prey species isotopes, see Mumby et al. (2018).

SIBER niche metrics and trophic position

Trophic niche area (SEA_C) ranged from 0.6‰^2 for Chinook salmon to 1.4‰^2 for rainbow trout (Table 2). The trophic niche for rainbow trout was oriented vertically (wide range of $\delta^{15}\text{N}$) while the brown trout trophic niche was oriented horizontally (wide range of $\delta^{13}\text{C}$) (Fig. 2). Trophic niche overlap was detected between all species except for lake trout which had a distinct trophic niche (Table 2; Fig. 2). Among the remaining salmonids, brown trout had lower niche overlap (<50%) and a high degree of isotopic distinctness from all other salmonids (Table 2; Fig. 2). The trophic niche for Chinook salmon was the smallest and thus did not strongly overlap other species with larger niche breadths, however, its niche was not very distinct and was strongly overlapped (>74%) by all species except lake trout and brown trout (Table 2; Fig. 2). The trophic niche for Atlantic salmon was similar to and strongly overlapped with Chinook salmon (96%) and coho salmon (75%), while coho salmon strongly overlapped with Atlantic salmon (65%) and Chinook salmon (81%) (Table 2; Fig. 2). Rainbow trout was similar to and overlapped with all three salmon (62–74%) but was relatively distinct from all salmonids (<50%) (Table 2; Fig. 2). Similar to trophic niche overlap, the trophic positions (TPs) of Lake Ontario salmonids were relatively the same (3.8–4.0), with the exception of lake trout exhibiting a slightly higher trophic position (4.4) (Table 2).

Salmonid diets based on stable isotope ratios and stomach contents

Stable isotope mixing model analyses using SIAR estimated that adult Lake Ontario salmonids consumed alewife as the most common prey type with dietary proportions ranging from 0.46 (lake trout) to 0.95 (rainbow trout; Table 3). Alewife was also the most common prey item, based on volume, within the stomach contents (Table 3). Round goby contributed between 0.03 (rainbow trout) to 0.26 (brown trout) of the diet based on mixing models (Table 3). Both stable isotope ratios and stomach contents suggest that to some extent rainbow smelt are consumed by lake trout, Chinook salmon and coho salmon (found in stable isotope ratios only), with both methods showing similar dietary proportions (albeit higher proportions for stable isotope ratios) for all species (Table 3).

Among the sculpin species, only slimy sculpin was found in lake trout stomachs by an extremely low proportion although stable isotope mixing models suggest sculpin are consumed more frequently (0.21 combined for both species) by lake trout. The other salmonids generally were not estimated to consume sculpin with mean dietary proportions from mixing models ranging between 0.00 and 0.04 (combined for both species; Table 3).

Salmonid diet and niche by size class

Values of $\delta^{13}\text{C}$ were significantly negatively correlated with fork length for Chinook salmon, coho salmon, lake trout and rainbow trout, and values of $\delta^{15}\text{N}$ were significantly positively correlated with fork length for brown trout and rainbow trout (Table 4). Mixing models indicated that consumption of alewife increased and round goby decreased in the Chinook salmon diet with increasing size (Table 5). The Chinook salmon trophic niche area remained unchanged with increasing length (Table 5). Samples sizes were deficient for coho salmon in most size classes and although $\delta^{13}\text{C}$ was negatively correlated with fork length, prey proportions did not vary greatly between the two size classes presented; however, the SEA_C greatly decreased with increasing size class for coho salmon (Table 5). The dietary proportion of rainbow smelt remained relatively consistent with increasing lake trout size class. Alewife and round goby did not have a consistent pattern with increasing lake trout size class, however, when alewife increased in dietary proportion, round goby would decrease and vice versa (Table 5). The SEA_C of lake trout fluctuated by size class (0.6 to 1.6) (Table 5). Rainbow trout appear to decrease its dietary proportion of alewife with

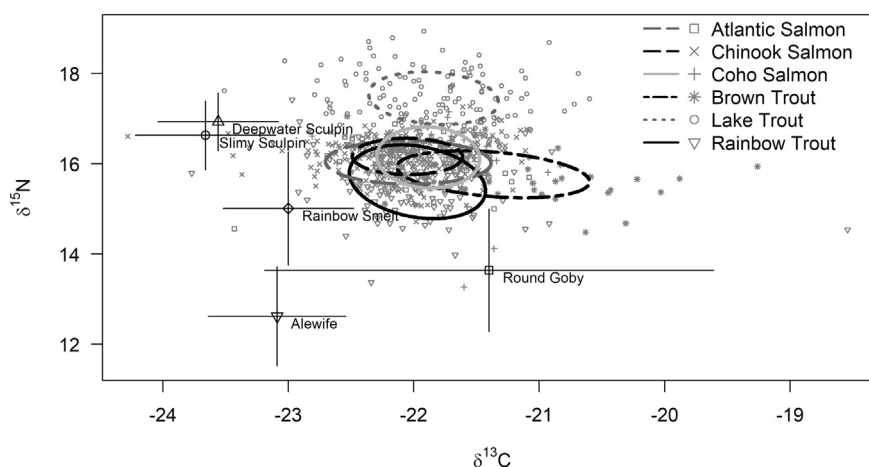


Fig. 2. Stable isotope bi-plot (isoscapes) of the isotopic niches of Lake Ontario salmonids and mean \pm standard deviation of lipid corrected stable isotope ratios of prey collected in 2013. Atlantic salmon collected from 2008 to 2011 and in 2013. Thick circles enclose standard (40%) ellipse areas (SEA_C) for all species with Atlantic salmon represented by a long dashed grey circle, Chinook salmon by a long dashed black circle, coho salmon by a solid light grey circle, brown trout by a dot dashed black circle, lake trout by a dotted dashed grey circle and rainbow trout by a solid black circle. Individual data points (light grey) are represented by squares for Atlantic salmon, x's for Chinook salmon, crosses for coho salmon, stars for brown trout, circles for lake trout, and inverted triangles for rainbow trout.

Table 2
Overlap in isotopic niche (%) based on SEAC and calculated trophic position (TP) for each salmonid species collected from Lake Ontario in 2013. Atlantic salmon collected from 2008 to 2011 and in 2013. Rows represent how similar species are with another species (e.g., Atlantic salmon covers 96% of the Chinook salmon niche) while columns represent how distinct species are from one another (e.g., only 53% of the Atlantic salmon niche overlaps with Chinook salmon, therefore 47% of the Atlantic salmon niche width is distinct from Chinook salmon). Niche area and SEAC (standard error of ellipse area) in %².

Species	Atlantic salmon	Chinook salmon	Coho salmon	Brown trout	Lake trout	Rainbow trout	SEAC (% ²)	Trophic position (TP)
Atlantic salmon	–	96	75	35	0	50	1.0	4.0
Chinook salmon	53	–	52	17	0	30	0.6	4.0
Coho salmon	65	81	–	34	0	40	0.9	4.0
Brown trout	42	36	47	–	0	33	1.2	3.9
Lake trout	0	0	0	0	–	0	0.9	4.4
Rainbow trout	67	74	62	37	0	–	1.4	3.8

increasing size class and increase the consumption, albeit a small amount, of all other prey items. With only two size classes with sufficient available data (400 to 499, $n = 17$ and 500 to 599 mm, $n = 13$), brown trout appear to have dietary proportions with more alewife and less round goby with increasing size, similar to Chinook salmon (Table 5).

Table 3
Estimated diet of Lake Ontario salmonids based on stomach contents (prey proportion based on relative weight of stomach contents) and estimated prey item contributions [mean (95% Bayesian credible interval)] via mixing models in SIAR.

Species	Prey item	Stomach content	Estimated prey item proportions
Atlantic salmon (Stomach $n = 0$) (Isotope $n = 41$)	Alewife	N/A	0.84 (0.76, 0.93)
	Rainbow smelt	N/A	0.04 (0.00, 0.11)
	Round goby	N/A	0.07 (0.00, 0.15)
	Deepwater sculpin	N/A	0.02 (0.00, 0.05)
	Slimy sculpin	N/A	0.02 (0.00, 0.06)
	Other	N/A	–
Chinook salmon (Stomach $n = 46$) (Isotope $n = 289$)	Alewife	0.98	0.83 (0.80, 0.87)
	Rainbow smelt	0.02	0.10 (0.05, 0.15)
	Round goby	0.00	0.05 (0.02, 0.08)
	Deepwater sculpin	0.00	0.01 (0.00, 0.03)
	Slimy sculpin	0.00	0.01 (0.00, 0.03)
	Other	0.00	–
Coho salmon (Stomach $n = 7$) (Isotope $n = 42$)	Alewife	1.00	0.80 (0.72, 0.88)
	Rainbow smelt	0.00	0.06 (0.00, 0.13)
	Round goby	0.00	0.10 (0.03, 0.17)
	Deepwater sculpin	0.00	0.02 (0.00, 0.05)
	Slimy sculpin	0.00	0.02 (0.00, 0.06)
	Other	0.00	–
Brown trout (Stomach $n = 27$) (Isotope $n = 47$)	Alewife	0.96	0.71 (0.60, 0.82)
	Rainbow smelt	0.00	0.01 (0.00, 0.04)
	Round goby	0.04	0.26 (0.15, 0.37)
	Deepwater sculpin	0.00	0.01 (0.00, 0.02)
	Slimy sculpin	0.00	0.01 (0.00, 0.02)
	Other	0.00	–
Lake trout (Stomach $n = 137$) (Isotope $n = 127$)	Alewife	0.61	0.46 (0.40, 0.52)
	Rainbow smelt	0.11	0.14 (0.00, 0.28)
	Round goby	0.26	0.19 (0.14, 0.25)
	Deepwater sculpin	0.00	0.09 (0.00, 0.20)
	Slimy sculpin	0.01	0.12 (0.00, 0.22)
	Other	0.01	–
Rainbow trout (Stomach $n = 21$) (Isotope $n = 126$)	Alewife	0.93	0.95 (0.91, 0.99)
	Rainbow smelt	0.00	0.01 (0.00, 0.02)
	Round goby	0.05	0.03 (0.00, 0.07)
	Deepwater sculpin	0.00	0.00 (0.00, 0.01)
	Slimy sculpin	0.00	0.00 (0.00, 0.01)
	Other	0.02	–

Note: “other” includes invertebrates (terrestrial, pelagic and benthic). Stomachs containing solely chyme and unidentified fish remains were removed from the analysis, along with empty stomachs which were also removed and not recorded in the sample numbers (n).

Discussion

Lake Ontario salmonid species had similar trophic positions and overlapping isotopic niches suggesting high potential for competition for dietary resources. Lake trout was the exception, as its isotopic niche did not overlap with the other salmonids and had an apparently higher trophic position. Little is known about the ecology of Atlantic salmon in Lake Ontario, but we found high isotopic niche overlap with other species, raising concerns about restoration potential for this species given the large population sizes of the other salmonid species. Lake-wide diet estimates, based on stable isotope ratios and stomach contents, showed that alewife was found in the highest proportions in all the salmonids, with lesser proportions of round goby and rainbow smelt, and negligible proportions of sculpin except in lake trout. This suggests that while some Lake Ontario salmonid species have incorporated round goby into their diets, alewife is the dominant prey species among Lake Ontario salmonids, as expected from previous studies.

Salmonid diet estimates showed agreement between stomach contents and stable isotope mixing model methods, identifying the same most common diet items (i.e., alewife) and giving relatively similar proportions. The mixing model method produced a wider range of prey items for all five species than stomach contents. The differences between the methods reflect either differences in the time captured or limitations associated with the two methods. Turnover time for ¹³C and ¹⁵N in muscle tissue of fish the size of those sampled here would be >6 months (especially larger, slower growing individuals), covering a much longer time period than stomach contents. If there are seasonal differences in the feeding behavior of the salmonids, then the stable isotope method may be reflecting this difference; however, given that samples were collected over a range of eight months the overall isotope signature for the species likely represents a combination of seasons, particularly in larger specimens. Investigating stomach contents could help discern any seasonal differences. Alternatively, variation in the isotopic values of prey items (e.g., changes with increasing size, or different location of capture) were not incorporated into the isotopic mixing models and might also explain the difference between the methods (Phillips et al., 2014). However, Harvey et al. (2002) found that although seasonal isotopic variation at the base of the food web can be propagated through different sizes of prey and predators, these signals are dampened with larger fish eating larger prey, such as the adult salmonids investigated in this study. Our isotopic mixing model had relatively small credible intervals and results were generally consistent with what is known about the feeding behavior of these species, which provides confidence in the isotope model reflecting more long-term feeding behavior. One of the limitations of the mixing model is that it creates dietary proportions only for prey included in the model (Phillips et al., 2014). If prey items that may be consumed are not included in the model, the model cannot accurately represent the true diet. However, based on past studies, and our observations of stomach contents for these species, we incorporated all major prey fish likely to be consumed. For instance, Johannsson et al. (2001) reported stable isotope ratios of *Mysis relicta* in Lake Ontario that would not be consistent with consumption by

Table 4

Linear regression analysis of stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) values against fork length = FL (mm) for each salmonid species collected in Lake Ontario. p-Values ≤ 0.05 (i.e., significant) are shown with regressions. The equation is as follows $\delta X = \beta \times \text{FL} \pm \alpha$, where α = intercept and β = slope.

Species	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$			
	α	β	R ²	p-Value	α	β	R ²	p-Value
Atlantic salmon	N/A	N/A	0.023	0.34	N/A	N/A	0.050	0.16
Chinook salmon	-21.154	-0.0013	0.342	<0.001	N/A	N/A	0.001	0.52
Coho salmon	-20.579	-0.0022	0.196	<0.01	N/A	N/A	0.006	0.61
Brown trout	N/A	N/A	0.034	0.22	14.567	0.0023	0.217	<0.001
Lake trout	-21.227	-0.001	0.053	<0.01	N/A	N/A	0.005	0.43
Rainbow trout	-21.018	-0.0016	0.077	<0.01	12.153	0.0058	0.446	<0.001

salmonids (outside the DTFD range, particularly for $\delta^{13}\text{C}$). We did not include invertebrate prey, which may have biased mixing model results for rainbow trout based on their isotopic niche. Future studies could assess the mixing model sensitivity by including more or fewer prey items, and the impact that has on the estimated prey proportions.

Alewife, as hypothesized, was the predominant prey item consumed (>80%) by five of the six salmonid species, based on both stomach contents and isotope mixing models, and likely accounted for the high isotopic niche overlap and similar trophic positions among the species; the exception was lake trout. Even for lake trout, alewife was the most common prey item based on stomach contents (0.72) and isotopic mixing models (0.46). In a previous isotope study of Lake Ontario salmonids, Yuille et al. (2015) also suggested a common prey source was driving the high isotopic overlap of salmonids in Lake Ontario. Thus, the preference for alewife in the diets of Lake Ontario salmonids has changed little since the 1980's (Brandt, 1986), which may be due to the large abundance of this prey species relative to other offshore prey species (Connerton et al., 2009).

Round goby was the second most consumed prey species based on stable isotope ratios and the presence in stomach contents of lake, brown, and rainbow trout. No round goby were found in the stomachs of Chinook and coho salmon (note, no Atlantic salmon stomach content data was available), but they were identified as a prey species by the stable isotope method. Rainbow smelt were observed in the stomach of Chinook salmon and lake trout at very low levels, and were identified in all species using stable isotope mixing models. Historically, rainbow smelt were a common prey of these salmonid species (Brandt, 1986). This change in prey consumption is likely due to a combination of the large decline of Lake Ontario rainbow smelt population (Holden and Connerton, 2015) and the invasion of round goby (Mills et al., 2003). Predation on round goby may occur during the spring before salmonids migrate to preferred cooler, offshore waters for the summer (Haynes et al., 1986; Scott and Crossman, 1998; Stewart and Bowlby, 2009), which may explain the difference between stomach content and isotopic mixing model methods. Colborne et al. (2016) utilized a third stable isotope ($\delta^{34}\text{S}$) to more accurately estimate lake trout feeding on round

Table 5

Stable isotopes ratios (mean \pm SE), standard ellipse area, and estimated diet of Lake Ontario salmonids via mixing models in SIAR [mean (95% Bayesian credible interval)] of salmonid species collected from Lake Ontario between April to December 2013. Atlantic salmon collected from 2008 to 2011, and 2013. Predator samples were lipid extracted.

Species	Length Class (mm)	n	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	SEAc (% ²)	Estimated prey item proportions				
			Mean \pm SE	Mean \pm SE		Alewife	Rainbow smelt	Round goby	Deepwater sculpin	Slimy sculpin
Atlantic salmon	400 to 499	5	16.1 \pm 0.2	-22.0 \pm 0.2	0.6	-	-	-	-	-
	500 to 599	31	15.9 \pm 0.1	-22.1 \pm 0.1	1.1	0.84 (0.75, 0.94)	0.04 (0.00, 0.11)	0.07 (0.00, 0.16)	0.02 (0.00, 0.06)	0.02 (0.00, 0.06)
	600 to 699	4	16.2 \pm 0.2	-21.9 \pm 0.3	0.3	-	-	-	-	-
	700 to 799	1	16.9	-22.2	N/A	-	-	-	-	-
Chinook salmon	300 to 399	33	16.3 \pm 0.1	-21.5 \pm 0.1	0.4	0.66 (0.57, 0.75)	0.08 (0.00, 0.16)	0.22 (0.15, 0.29)	0.02 (0.00, 0.05)	0.02 (0.00, 0.06)
	400 to 499	38	16.2 \pm 0.1	-21.8 \pm 0.1	0.4	0.74 (0.66, 0.82)	0.08 (0.00, 0.15)	0.14 (0.07, 0.21)	0.02 (0.00, 0.05)	0.02 (0.00, 0.06)
	500 to 599	19	16.0 \pm 0.1	-21.9 \pm 0.1	0.4	0.78 (0.67, 0.88)	0.07 (0.00, 0.16)	0.11 (0.01, 0.20)	0.03 (0.00, 0.07)	0.03 (0.00, 0.08)
	600 to 699	36	16.0 \pm 0.1	-22.0 \pm 0.1	0.4	0.82 (0.74, 0.90)	0.07 (0.00, 0.15)	0.06 (0.00, 0.12)	0.02 (0.00, 0.06)	0.03 (0.00, 0.07)
	700 to 799	35	16.1 \pm 0.1	-22.2 \pm 0.1	0.4	0.82 (0.74, 0.90)	0.08 (0.00, 0.17)	0.03 (0.00, 0.08)	0.03 (0.00, 0.08)	0.04 (0.00, 0.09)
	800 to 899	83	16.2 \pm 0.0	-22.2 \pm 0.0	0.4	0.84 (0.79, 0.89)	0.08 (0.00, 0.15)	0.01 (0.00, 0.04)	0.03 (0.00, 0.07)	0.04 (0.00, 0.09)
	900 +	45	16.2 \pm 0.1	-22.3 \pm 0.1	0.5	0.84 (0.77, 0.91)	0.06 (0.00, 0.14)	0.02 (0.00, 0.06)	0.04 (0.00, 0.09)	0.05 (0.00, 0.11)
Coho salmon	400 to 499	5	16.0 \pm 0.2	-21.8 \pm 0.2	0.4	-	-	-	-	-
	500 to 599	18	16.0 \pm 0.2	-21.7 \pm 0.1	1.2	0.70 (0.50, 0.87)	0.08 (0.00, 0.21)	0.16 (0.05, 0.27)	0.03 (0.00, 0.09)	0.03 (0.00, 0.09)
	600 to 699	17	16.3 \pm 0.1	-22.0 \pm 0.1	0.2	0.73 (0.61, 0.85)	0.10 (0.00, 0.23)	0.08 (0.00, 0.16)	0.04 (0.00, 0.11)	0.05 (0.00, 0.12)
	700 to 799	1	16.0	-22.6	N/A	-	-	-	-	-
	800 to 899	0	N/A	N/A	N/A	-	-	-	-	-
	900 +	1	16.0	-22.5	N/A	-	-	-	-	-
Brown trout	300 to 399	7	15.3 \pm 0.2	-21.1 \pm 0.3	1.0	-	-	-	-	-
	400 to 499	17	15.7 \pm 0.1	-21.3 \pm 0.2	1.1	0.65 (0.43, 0.86)	0.03 (0.00, 0.10)	0.28 (0.07, 0.47)	0.02 (0.00, 0.05)	0.02 (0.00, 0.05)
	500 to 599	13	15.8 \pm 0.1	-21.6 \pm 0.2	1.0	0.68 (0.45, 0.90)	0.05 (0.00, 0.15)	0.22 (0.02, 0.39)	0.03 (0.00, 0.07)	0.03 (0.00, 0.08)
	600 to 699	7	16.3 \pm 0.2	-21.7 \pm 0.3	1.3	-	-	-	-	-
	700 to 799	3	15.8 \pm 0.3	-21.0 \pm 0.6	3.3	-	-	-	-	-
Lake trout	300 to 399	13	17.6 \pm 0.1	-21.5 \pm 0.1	0.6	0.31 (0.18, 0.44)	0.21 (0.01, 0.39)	0.28 (0.18, 0.39)	0.10 (0.00, 0.23)	0.10 (0.00, 0.23)
	400 to 499	16	17.4 \pm 0.2	-21.8 \pm 0.2	1.6	0.39 (0.22, 0.56)	0.18 (0.00, 0.39)	0.25 (0.08, 0.40)	0.09 (0.00, 0.21)	0.10 (0.00, 0.23)
	500 to 599	22	17.5 \pm 0.1	-22.0 \pm 0.1	0.8	0.47 (0.34, 0.59)	0.18 (0.00, 0.38)	0.12 (0.02, 0.23)	0.10 (0.00, 0.23)	0.12 (0.00, 0.25)
	600 to 699	58	17.4 \pm 0.1	-21.9 \pm 0.1	0.7	0.47 (0.38, 0.55)	0.18 (0.02, 0.35)	0.16 (0.11, 0.22)	0.09 (0.00, 0.19)	0.10 (0.00, 0.20)
	700 to 799	17	17.4 \pm 0.2	-21.9 \pm 0.2	1.3	0.42 (0.26, 0.58)	0.19 (0.00, 0.38)	0.20 (0.03, 0.35)	0.09 (0.00, 0.21)	0.10 (0.00, 0.24)
	800 to 899	1	18.3	-23.0	N/A	-	-	-	-	-
Rainbow trout	300 to 399	3	12.8 \pm 1.2	-20.6 \pm 1.1	22.7	-	-	-	-	-
	400 to 499	9	15.0 \pm 0.2	-21.9 \pm 0.1	0.4	-	-	-	-	-
	500 to 599	58	15.5 \pm 0.1	-22.0 \pm 0.1	0.8	0.93 (0.89, 0.98)	0.01 (0.00, 0.04)	0.04 (0.00, 0.08)	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)
	600 to 699	39	16.0 \pm 0.1	-22.0 \pm 0.1	0.7	0.86 (0.78, 0.93)	0.04 (0.00, 0.10)	0.07 (0.01, 0.13)	0.02 (0.00, 0.04)	0.02 (0.00, 0.05)
	700 to 799	14	16.1 \pm 0.1	-22.1 \pm 0.1	0.6	0.78 (0.64, 0.90)	0.08 (0.00, 0.20)	0.06 (0.00, 0.14)	0.04 (0.00, 0.10)	0.04 (0.00, 0.11)
	800 to 899	3	17.1 \pm 0.4	-22.4 \pm 0.4	0.3	-	-	-	-	-

goby, and similarly investigating dietary preferences with the addition of $\delta^{34}\text{S}$, or alternative analyses such as using fatty acids or compound-specific stable isotopes, may further discern the extent of round goby consumption by all salmonids. Sculpin species were only identified in the diet of lake trout, consistent with a deeper water preference by this salmonid.

Chinook and coho salmon, and in part Atlantic salmon had high trophic niche overlap, relative to the other salmonids, with low $\delta^{13}\text{C}$ values suggestive of primarily feeding within the pelagic offshore of Lake Ontario. This is consistent with stomach contents and provides further evidence that these species feed almost exclusively on alewife, and that there is little variation in diet between individuals. Chinook and coho salmon had been documented to feed primarily on alewife in Lake Ontario (Brandt, 1986). Chinook salmon in particular, are considered an obligate pelagic predator by not changing foraging strategies with changes in prey abundance (Diana, 1990; Jacobs et al., 2013; Roseman et al., 2014). While coho salmon also had a small niche area, they may be more capable of feeding on other available pelagic prey than Chinook salmon (Roseman et al., 2014). As pelagic prey are depleted in ^{13}C relative to nearshore or benthic prey (France, 1995a, 1995b), there was also evidence of a small diet shift to more pelagic prey as Chinook and coho salmon become larger (decreasing $\delta^{13}\text{C}$ with increasing length). The mixing models indicated this dietary change with an increased consumption of round goby in smaller fish, however, this may be a spurious assignment as round goby was the species in the model with the highest $\delta^{13}\text{C}$ value. Other nearshore pelagic prey could also be consumed by smaller Chinook and coho that were not used in our mixing model. Alternatively, smaller alewife were found to have higher $\delta^{13}\text{C}$ values, utilizing more nearshore areas (Mumby et al., 2018), and may explain the shift in $\delta^{13}\text{C}$ in Chinook and coho salmon. As Chinook salmon size increased, so did the size of the alewife being consumed (Jacobs et al., 2013) which could influence the decrease in $\delta^{13}\text{C}$ of Chinook salmon seen here.

Rainbow trout had the largest niche area ($1.4\%{}^2$) of all the salmonids, with little variation in $\delta^{13}\text{C}$ suggesting individuals mainly feed in the pelagic zone but based on a broad $\delta^{15}\text{N}$ range are feeding at different trophic positions. More than 50% of the rainbow trout niche did not overlap with the other salmonids, thus some individuals feed at a lower trophic level and have a more diverse diet of prey than the other salmonids. Previous studies on stomach contents and stable isotope ratios of rainbow trout have shown a higher proportion of invertebrates (Brandt, 1986; Negus and Hoffman, 2013; Roseman et al., 2014) which is likely driving this trophic niche. There was a shift towards a more piscivorous diet or higher trophic position (increasing $\delta^{15}\text{N}$) with increasing rainbow trout size, consistent with the ontogenetic feeding of the species seen in other systems (Scott and Crossman, 1998). The lack of invertebrate isotope data may be compromising the mixing model results for rainbow trout, for example, a recent a study on lake trout using stable isotope ratios identified mysids as a minor diet item (Rush et al., 2012). However, invertebrates were hardly present in rainbow trout stomach contents in this study.

The largest isotopic niches occurred with brown trout and lake trout, likely due to more diverse diets as identified by the stomach contents and isotope methods. The orientation of the brown trout trophic niche (i.e., narrow $\delta^{15}\text{N}$ range but broad $\delta^{13}\text{C}$ range) suggested that this species fed across habitats, in the nearshore or benthic, and offshore pelagic. Olson et al. (1988) found that the majority of brown trout were caught in nearshore (≤ 30 m) waters during both the spring and summer seasons. Our results support this assumption as dietary proportions of round goby were highest in brown trout, particularly if they are spending more time in the nearshore than other salmonids where round goby reside in the spring and summer (Miller, 1986). However, the broad $\delta^{13}\text{C}$ range in brown trout could be indicative that they are feeding upon other nearshore prey that have high $\delta^{13}\text{C}$ values that were not included in the analyses. With round goby having the highest $\delta^{13}\text{C}$ value in our mixing model, it could artificially increase the dietary proportion

of round goby when other nearshore fish were potentially being consumed.

Lake trout did not exhibit an isotopic niche overlap with any other salmonid, despite the importance of alewife in all salmonid diets. Our mixing models indicated that though lake trout had a more diverse diet than brown trout, this species had a smaller isotopic niche at an apparently higher trophic level and suggests less variation in diet between individuals. Diet analysis (both stomach contents but more so mixing models) indicated a large percentage of the diet contained benthic fish (e.g., round goby and to some extent sculpin). Olson et al. (1988) found that lake trout abundance is much higher below the thermocline in Lake Ontario, and as such could be actively foraging on benthic prey. The elevated $\delta^{15}\text{N}$ and apparent higher trophic position of lake trout compared to other salmonids could be due to a few reasons. Lake trout consuming more benthic prey (e.g., sculpin and round goby), which have higher $\delta^{15}\text{N}$ values than alewife, could artificially elevating the trophic position of lake trout to the other salmonids. If the trophic position was calculated using the mean $\delta^{15}\text{N}$ of round goby (the most consumed benthic prey item in stomachs of lake trout) instead of alewife, the trophic position changes from 4.4 to 4.1, and is similar to the other salmonids. The high $\delta^{15}\text{N}$ may also be attributed to lake trout feeding in deeper waters than the other salmonids as $\delta^{15}\text{N}$ can increase with depth in lakes (Sierszen et al., 2014). Also, lake trout can be cannibalistic as well as eating other young salmonids (Dietrich et al., 2006; Madenjian et al., 1998; Roseman et al., 2014). Salmonids can contribute up to 10% of lake trout diets by mass (Madenjian et al., 1998; Roseman et al., 2014), and potentially influence the elevated ^{15}N of the lake trout and trophic position. Small salmonids were not included in the mixing model and therefore were not estimated in the dietary proportions for lake trout. Lake trout diet did not consistently vary between size classes; and although $\delta^{13}\text{C}$ increased with increasing length, it did not explain much of the variation ($R^2 = 0.053$). This suggests that lake trout >300 mm do not shift their trophic niche as they grow and consistently feed on a varied diet, both pelagic and benthic, available within the hypolimnion. Thus, lake trout may not actively have prey preference but simply consume prey they encounter in their habitat (Olson et al., 1988).

This is the first study to evaluate the trophic niche and diet of the Lake Ontario Atlantic salmon population, and thus no data are available to compare. Results suggest they heavily overlap in trophic niches and feed on similar items as Chinook and coho salmon, indicating an offshore signal and a diet dominated by alewife, with low proportions of round goby and rainbow smelt. This alewife-dominated diet of the Atlantic salmon raises the concern about the successful reintroduction of this species; stocking of Atlantic salmon into Lake Ontario has intensified in the past several years, yet few spawners appear in tributary streams (Stewart and Johnson, 2014). High consumption of alewives has been linked to the decline and eventual extirpation of the historic Lake Ontario Atlantic salmon population through anorexia and early mortality syndrome (EMS) via thiamine-deficiency (Ketola et al., 2000; Madenjian et al., 2008). Atlantic salmon appear to be more prone to developing thiamine-deficiency than other Great Lakes salmonids (Ketola et al., 2000; Ketola et al., 2005; Ketola et al., 2009). Thus, Atlantic salmon populations may potentially be constrained from competition for prey by non-native Chinook and coho salmon, or reduced survival (via thiamine-deficiency) from consuming primarily non-native alewife.

Overall, diet reconstruction detected that the consumption of alewife was causing the high trophic niche overlap between the salmonids in Lake Ontario. Chinook, coho and Atlantic salmon appear to be less variable in foraging strategies with overlapping and/or smaller niche breadths than the lake, brown and rainbow trout. Although we cannot attribute consumption estimates to dietary preferences or prey availability, there is indication that some salmonids (at least lake, brown, and rainbow trout, as also seen in stomach contents) are utilizing round goby in a minor capacity to supplement their diet. Thus, food

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